

1992

NASA/ASEE SUMMER FACULTY FELLOWSHIP PROGRAM

MARSHALL SPACE FLIGHT CENTER  
THE UNIVERSITY OF ALABAMA

GROUND TESTING OF BIOCONVECTIVE VARIABLES SUCH AS MORPHOLOGICAL  
CHARACTERIZATIONS AND MECHANISMS WHICH REGULATE MACROSCOPIC  
PATTERNS.

Prepared By:                   Adriel D. Johnson, Ph.D.

Academic Rank:               Assistant Professor

Institution and  
Department:                   University of Alabama in Huntsville  
                                  Department of Biological Sciences

NASA/MSFC:

    Office:                   Space Sciences Laboratory  
    Division:                Microgravity Sciences & Applications  
    Branch:                   Biophysics

MSFC Colleagues:             Helen C. Matsos  
                                  David A. Noever, Ph.D.



Conditions simulating low- and high-gravity, reveal changes in macroscopic pattern formation in selected microorganisms, but whether these structures are gravity dependent is not clear. Two theories have been identified in the fluid dynamics community which support macroscopic pattern formation. The first one is gravity dependent (fluid density models) where small concentrated regions of organisms sink unstably, and the second is gravity independent (wave reinforcement theory) where organisms align their movements in concert, such that either their swimming strokes beat in phase or their vortices entrain neighbors to follow parallel paths (3). Movement or locomotion of microorganisms such as paramecium, algae, protozoa, and spermatozoa, form macroscopic patterns analogous to thermally driven convection cells. Gravitational test have shown bioconvective pattern for both the flagellated algae, *Polytomella parva* and the ciliated non-xyrotaxic protozoa, *Tetrahymena pyriformis* to show similar polygonal morphology, changes in the inter-nodal distances, and decreases in pattern wavenumber and fineness (3). In addition, macroscopic patterns of caprine spermatozoa continue during conditions of variable gravity (1). Studies have shown that macroscopic pattern formation is consistent with the fluid density models for protozoa and algae and wave reinforcement hypothesis for caprine spermatozoa (1,3).

Nearly every eukaryotic cell contains microtubules including algae, protozoa, and spermatozoa. Microtubules are important cellular components essential for movement of the cytoskeleton, spindle fibers, cilia, and flagella. In cilia and flagella, microtubules are in a characteristic array of nine outer doublets of microtubules and two central single microtubules. All microtubules have a common ultrastructure composed of tubulin, a dimeric globular protein capable of generating various dissipative structures when combined with guanosine triphosphate in solution. These structures include traveling waves of microtubule assembly and disassembly and the formation of polygonal networks, suggesting dynamic spatial pattern formation of microtubule components in the absence of cellular organizing centers (2). Thus the microtubule assembly and disassembly process in vitro could serve as a ground base model by examining regulatory mechanisms for comparing pattern formation by selected organisms under microgravity conditions.

As a part of Marshall Space Flight Centers' Summer Faculty Fellowship Program within the Biophysics Branch, the objectives of the summer faculty fellow were to provide a procedure to isolate bovine tubulin, to assist with determining whether the microtubule assembly and disassembly system could be quantitated using spectrophotometric techniques for ground based studies, and to assist with the development of a technique to examine the microtubule assembly and disassembly system for future studies under the simulated microgravity conditions of the KC-135 experimental aircraft.

The brain tubulin preparation procedure was as follows: bovine brains were obtained from a local slaughter house, put on ice, meninges removed, and the cortex minced. Brains were washed with cold calcium free phosphate buffered saline solution and suspended in cold Hanks physiological buffer. The tissue was homogenized with a polytron and centrifuged at 14,000 rpm for 20 minutes at 5°C. The supernatant was collected and ultracentrifuged at 40,000 rpm for 1 hour at 5°C. Guanosine triphosphate (GTP) was added, incubated at 37°C for 45 minutes and centrifuged at 14,000 rpm for 40 minutes at 28°C. The pellet was resuspended with cold Hanks buffer, GTP added, homogenized with a Teflon-glass homogenizer, and centrifuged at 18,000 rpm for 30 minutes at 5°C. The mixture was allowed to polymerize at 37°C for 15-20 minutes, and the pellet can then be used for phosphocellulose purification of the tubulin.

Microtubules are the only biopolymers which can simultaneously polymerize and depolymerize (2). The tubulin preparation procedure described above provides a source of tubulin which can facilitate the study of the assembly and disassembly process of the microtubule system. Further understanding of the microtubule system would provide a data base for both ground base and microgravity studies on this regulatory mechanism associated to pattern formations in organisms.

#### References

1. Johnson, J.U. Biological patterns: Novel indicators for pharmacological assays. NASA Contractor Report - 184253, Research Reports - 1991 NASA/ASEE Summer Faculty Fellowship Program. R-R2. October 1991.
2. Mandelkow, E., Mandelkow, E.-M., Hotani, H., Hess, B. and Muller, S.C. Spatial patterns from oscillating microtubules. Science. 246:1291-1293. December 1989.
3. Noever, D.A., Matsos, H.C., Johnson, J.U., and Cronise, R.J. A gravitational test of bioconvective patterns: Wave reinforcement versus fluid density models (Submitted and personal communication) 1992.